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DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

R & D STATUS REPORT

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ULTRASENSITIVE DETECTION OF CHEMICAL SUBSTANCES

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QUARTERLY REPORT

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In our second quarter we have continued in our efforts to quantitate the relative binding constants of our monoclonal antibodies and in reducing noise factors. These two tasks are centrally important to our success and we will be devoting more time to them in the coming months.

During this quarter we were able to measure the intrinsic affinity constant of protein A purified antibody specific for AChE-DFP conjugate. The calculated affinity constant was 1.12×10^8 , mole⁻¹, which is a reasonably high affinity. Since affinity constants reflect the dissociation and association constant, and association constants will be similar for antibodies to a particular antigen, we can test our monoclonal antibodies for their relative strength of binding using affinity constant calculations. Thus, for the time being, we see no essential need to calculate dissociation constants directly.

Figure 1 represents OD data obtained by incubating 0.1 ml of 2-fold geometric dilutions of a 4.3×10^{-11} mole protein A purified Ab specific for AChE-DFP in microtiter plate wells containing bound AChE-DFP. The ODs were determined utilizing anti-mouse IgG-HRP. (Note that the Ab used for testing was not obtained from a single clone.) By determining the inflection points, the concentration of Ab at 50% saturation was determined.

$$\text{Since } K = \frac{[Ab \cdot Ag]}{[Ab][Ag]},$$

and when 50% saturation of Ag occurs $[Ab \cdot Ag] = [Ag]$.

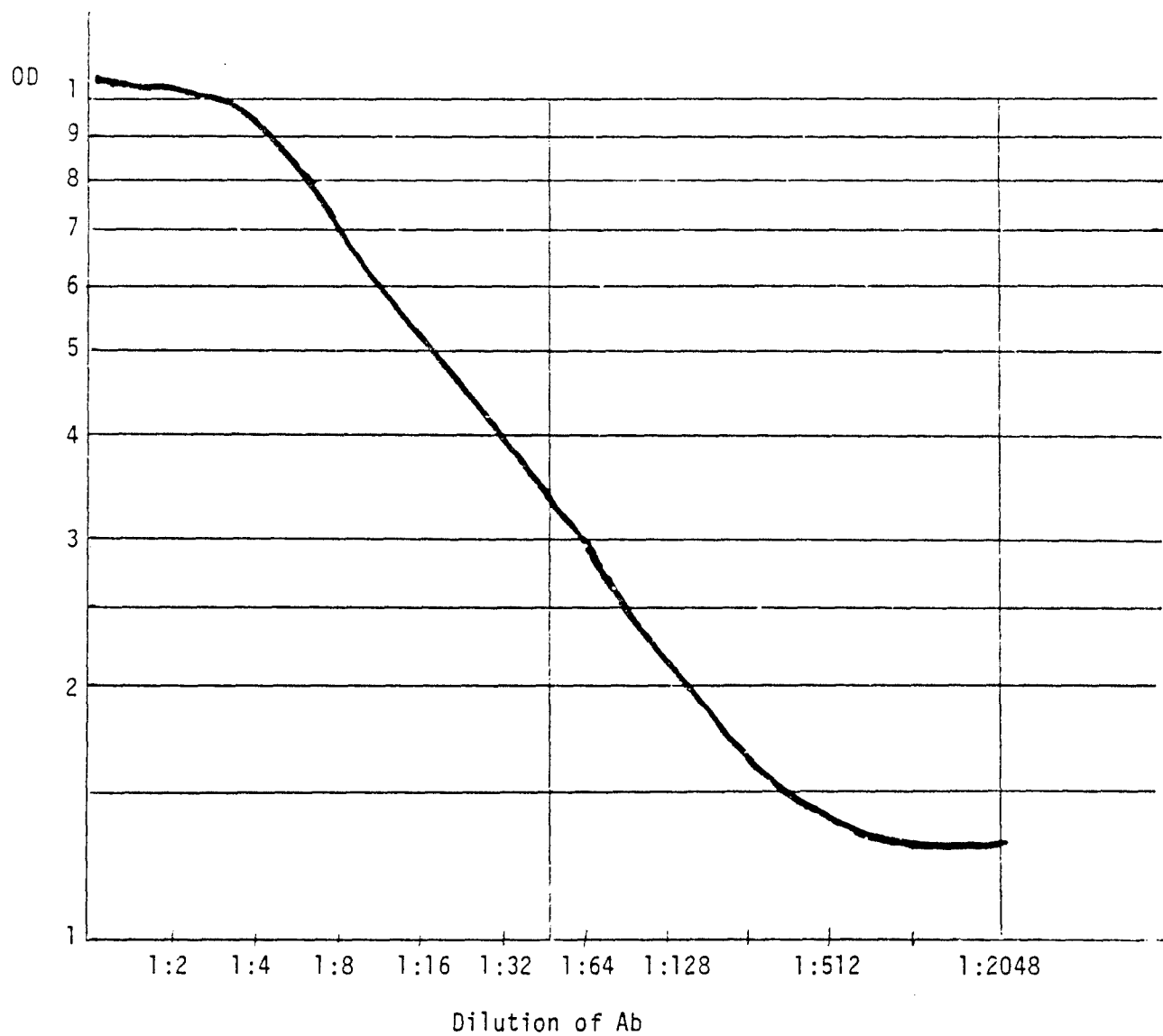
$$\text{Therefore, } K = \frac{1}{[Ab]}.$$

The Ab concentration at 50% Ag saturation is =

$$\frac{1}{48} (4.3 \times 10^{-11} \text{ mole}) = 8.95 \times 10^{-13} \text{ mole, or } 8.95 \times 10^{-9} \text{ M.}$$

$$\text{Therefore, } K = \frac{1}{8.95 \times 10^{-9} \text{ M}} = 1.12 \times 10^8.$$

FIGURE 1



As monoclonal antibodies continue to be produced, they will be selected for high affinity constants. This effort will require significant numbers of monoclonal antibodies and is important to our ability to stabilize the antigen/antibody bond.



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